New Diagnostic Methods for Tuberculosis

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Foundation for Innovative New Diagnostics
Disclosure

FIND has contractual relationships with a number of companies whose products are mentioned in this presentation. These agreements call for FIND to support development work and undertake studies of their TB diagnostics assays. In turn, these companies are to provide their tests at favorable prices to the public sector in developing countries.
FIND’s Diagnostic Pipeline
Manual Solution: Eiken

- Closed system
- Isothermal
- Rapid
- Multiprimer
- Visible readout

Loop-mediated Isothermal Amplification (LAMP)

Target DNA

EIKEN CHEMICAL CO., LTD.
## Clinical Performance

<table>
<thead>
<tr>
<th></th>
<th>Sensitivity in ss+, LJ+</th>
<th>Sensitivity in ss-, LJ+</th>
<th>Specificity in LJ-</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lima</td>
<td>97.7% (75/78)</td>
<td>51.8% (14/27)</td>
<td>99.3% (152/153)</td>
</tr>
<tr>
<td>Dhaka</td>
<td>98.4% (61/62)</td>
<td>50% (2/4)</td>
<td>97.8% (181/185)</td>
</tr>
<tr>
<td>Mbeya</td>
<td>100% (37/37)</td>
<td>41.7% (5/12)</td>
<td>100% (167/167)</td>
</tr>
<tr>
<td>Total</td>
<td>97.7% (173/177)</td>
<td>48.8% (21/43)</td>
<td>99.0% (500/505)</td>
</tr>
</tbody>
</table>

“The assay was **robust**, with high end-point stability and low rates of test failure. **Technicians with no prior molecular experience easily performed the assay after 1 week of training**, and opportunities for further simplification of the assay were identified”.

Development Progress for TB LAMP: V.1.0 – V.1.1

- From 4 to 2 instrument
- From 32 to 28 steps
- From 15 to 10 plastic devices
- From 55 to 40 min hands on time per run
Hain GenoType® MTBDRPlus Test
Background

• In response to M/XDR TB crisis in southern Africa, FIND is undertaking demonstration studies of new technologies for rapid MDR TB screening
• The largest of these is underway in South Africa and includes an assessment of rapid screening with the GenoType® MTBDRplus assay in 20,000 patients at risk of MDR TB in 4 provinces
• Prior to enrollment of patients into the demonstration project, the assay was validated in two NHLS laboratories
GenoType® MTBDRplus Assay

- PCR test that detects *M. tuberculosis* and genetic mutations conferring rifampin and isoniazid resistance directly from AFB smear-positive sputum specimens
- Three published studies of earlier generation of the assay indicated good performance on AFB+ sputum
- Second generation assay includes additional probe for INH-R and more sensitive amplification control
- One published study from NRL-Germany on 72 AFB+ sputum suggest high sensitivity and specificity for MDR TB
GenoType® MTBDRplus test procedure

1) DNA Extraction
   From NALC/NaOH Processed sputum

2) Amplification by PCR

3) Hybridization
   Reverse hybridization of amplified nucleic acids to specific DNA probes bound on strips

4) Evaluation
GenoType® MTBDRplus
Rifampicin resistance: *rpoB* gene

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rpoB wildtype-probes: WT1 to WT8
rpoB mutation probes: MUT1-3: D516V, H526Y, H526D, S531L
→ Detection of mutations through missing of wildtype signals
→ Detection of mutations through presence of mutation signals

---

GenoType® MTBDRplus
Rifampicin resistance: *rpoB* gene

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→ Detection of mutations through missing of wildtype signals
→ Detection of mutations through presence of mutation signals
### GenoType® MTBDRplus

#### Isoniazid resistance: katG gene

<table>
<thead>
<tr>
<th>missing wild type probe</th>
<th>analyzed codon</th>
<th>mutation probe</th>
<th>mutation</th>
</tr>
</thead>
<tbody>
<tr>
<td>katG WT</td>
<td>315</td>
<td>katG MUT1</td>
<td>S315T1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>katG MUT2</td>
<td>S315T2</td>
</tr>
</tbody>
</table>

Mutations in the katG gene and the corresponding wild type and mutation probes

### GenoType® MTBDRplus

#### Isoniazid resistance: inhA gene

<table>
<thead>
<tr>
<th>missing wild type probe</th>
<th>analyzed nucleic acid position</th>
<th>mutation probe</th>
<th>mutation</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>inhA</em> WT1</td>
<td>−15</td>
<td><em>inhA</em> MUT1</td>
<td>C15T</td>
</tr>
<tr>
<td></td>
<td>−16</td>
<td><em>inhA</em> MUT2</td>
<td>A16G</td>
</tr>
<tr>
<td><em>inhA</em> WT2</td>
<td>−8</td>
<td><em>inhA</em> MUT3A</td>
<td>T8C</td>
</tr>
<tr>
<td></td>
<td></td>
<td><em>inhA</em> MUT3B</td>
<td>T8A</td>
</tr>
</tbody>
</table>

Mutations in the inhA promotor region and the corresponding wild type and mutation probes
Reaction zones of GenoType® MTBDRplus (examples)

- Conjugate Control (CC)
- Amplification Control (AC)
- M. tuberculosis complex (TUB)

**rpoB Locus Control**
- rpoB wild type probe 1 [rpoB WT1]
- rpoB wild type probe 2 [rpoB WT2]
- rpoB wild type probe 3 [rpoB WT3]
- rpoB wild type probe 4 [rpoB WT4]
- rpoB wild type probe 5 [rpoB WT5]
- rpoB wild type probe 6 [rpoB WT6]
- rpoB wild type probe 7 [rpoB WT7]
- rpoB wild type probe 8 [rpoB WT8]
- rpoB mutation probe 1 [rpoB MUT1]
- rpoB mutation probe 2A [rpoB MUT2A]
- rpoB mutation probe 2B [rpoB MUT2B]
- rpoB mutation probe 3 [rpoB MUT3]

**katG Locus Control**
- katG wild type probe [katG WT]
- katG mutation probe 1 [katG MUT1]
- katG mutation probe 2 [katG MUT2]

**inhA Locus Control**
- inhA wild type probe 1 [inhA WT1]
- inhA wild type probe 2 [inhA WT2]
- inhA mutation probe 1 [inhA MUT1]
- inhA mutation probe 2 [inhA MUT2]
- inhA mutation probe 3A [inhA MUT3A]
- inhA mutation probe 3B [inhA MUT3B]

<table>
<thead>
<tr>
<th>Resistance</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
</tr>
</thead>
<tbody>
<tr>
<td>R</td>
<td></td>
<td></td>
<td>R+I</td>
<td></td>
<td>R+I</td>
</tr>
<tr>
<td>I</td>
<td></td>
<td>R+I</td>
<td>I</td>
<td>R+I</td>
<td>R+I</td>
</tr>
</tbody>
</table>

R = Rifampicin, I = Isoniazid

**Find Diagnostics**
NHLS Cape Town Evaluation Study*

- MTBDRplus assay performed on 536 consecutive AFB+ sputum specimens submitted between 31 Jan and 1 Mar 2007
- Specimens cultured on MGIT and DST on 7H11 (proportion method)
- 100 AFB- specimens also tested
- Primary outcome: performance/test accuracy for INH-R, RIF-R and MDR TB on AFB+ specimens compared to conventional DST
- Secondary outcomes: TAT, performance on AFB- specimens

Results of MTBDRplus Testing

- 97% of specimens had interpretable results within 1-2 days (TAT < 7 days)
- 55 MGIT cultures were contaminated
- 86 MDR isolates by conventional testing
- Sensitivity, specificity, PPV, and NPV for:
  - RIF-R: 99%, 99%, 98%, 100%
  - INH-R: 94%, 100, 99%, 98%
  - MDR TB: 99%, 100%, 100%, 100%
- Accurate results for 14/15 AFB-/cult+ sputa
Conclusions

• Overall performance of MTBDRplus assay is superior to conventional culture/DST: speed, accuracy, interpretable results, high throughput
• Cost of testing may be less than culture/DST
• Can substantially reduce the need for culture/DST when screening for MDR TB
• May be much easier to establish than new culture-capable laboratory
• New sputum processing methods may both increase sensitivity and facilitate transport
FIND-MRC-NHLS South Africa Demonstration Project
South Africa Demonstration Project

- **Aim:** Assess the use of MTBDR\textit{plus} for diagnosis of MDR-TB under operational (NTP) conditions in South Africa, a high TB and HIV burden low-middle income country
- Prospective cohort of cases/suspects at high risk for MDR-TB
- Four provinces, each with one referral laboratory performing rapid assays
- MTBDR\textit{plus} result compared with gold standard (MGIT culture + DST)
- 20 000 sputum specimens from high risk MDR-TB suspects
- Patient management based on rapid assay result
- Conventional DST remains gold standard if discrepancy with rapid assay result
Results (preliminary report for DSMB, Feb 2008)

- 92.0% (2032/2208) results were valid by MTBDR\textit{plus} assay
- 77.5% (1712/2208) results were valid by MGIT culture and conventional DST (either MGIT DST or 7H11 proportion method)
- No significant difference in the proportion of valid MTBDR\textit{plus} results directly from sputum or from positive MGIT cultures
- MTBDR\textit{plus} gave results in 399 specimens with no DST result (84%), 31 were MDR-TB
Performance of MTBDR*plus*, all specimens (n=2246)

<table>
<thead>
<tr>
<th></th>
<th>Rifampicin</th>
<th>Isoniazid</th>
<th>Multidrug-resistance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sensitivity, %</td>
<td>98.4%</td>
<td>91.4%</td>
<td>96.5%</td>
</tr>
<tr>
<td>(95% CI)</td>
<td>(95.3 – 99.7)</td>
<td>(87.2 – 94.6)</td>
<td>(92.0 – 98.9)</td>
</tr>
<tr>
<td>Specificity, %</td>
<td>99.1%</td>
<td>99.7%</td>
<td>99.7%</td>
</tr>
<tr>
<td>(95% CI)</td>
<td>(98.5 – 99.5)</td>
<td>(99.3 – 99.9)</td>
<td>(99.2 – 99.9)</td>
</tr>
<tr>
<td>Overall accuracy, %</td>
<td>99.0%</td>
<td>98.4%</td>
<td>99.4%</td>
</tr>
<tr>
<td>(95% CI)</td>
<td>(98.5 – 99.5)</td>
<td>(97.8 – 99.0)</td>
<td>(98.9 – 99.7)</td>
</tr>
<tr>
<td>PPV (95% CI)</td>
<td>93.2% (88.7 – 96.3)</td>
<td>98.2% (95.6 – 99.5)</td>
<td>96.5% (92.0 – 98.9)</td>
</tr>
<tr>
<td>NPV (95% CI)</td>
<td>99.8% (99.4 – 100.0)</td>
<td>98.5% (97.7 – 99.1)</td>
<td>99.7% (99.2 – 99.9)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>MGIT + conventional DST</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>MTBDR*plus</strong></td>
<td>MDR</td>
</tr>
<tr>
<td>MDR</td>
<td>139</td>
</tr>
<tr>
<td>Not MDR</td>
<td>5</td>
</tr>
<tr>
<td>Total</td>
<td>144</td>
</tr>
</tbody>
</table>
MDR discrepant results

- 5 false negatives for MDR
  - 4/5 were RIF resistant by MTBDRplus

- 5 false positives for MDR
  - 1 isolate: rpoB S531L + inhA -15C15T
  - 2 isolates: rpoB mut 526 – 529 + katG S315T1
  - 2 isolates: rpoB mut 516-519 + katG S315T1 (1 isolate also had mut @ 513-517: possible Del515, D526V or D516Y)
Turnaround time (TAT)

TAT from receipt of specimens at the laboratory to reporting of PCR result, Cape Town

TAT from specimen collection to receipt at lab was 2 days (range 0-15 days)
TAT of MGIT culture and DST was median of 41 days (range 21 to 69 days)
Cost of MTBDR<em>plus</em> vs MGIT culture + DST

Costs of performing MTBDR<em>plus</em> assay in Cape Town and Johannesburg laboratories

<table>
<thead>
<tr>
<th>Location</th>
<th>Decon.</th>
<th>Auramine</th>
<th>MDRTB&lt;em&gt;plus&lt;/em&gt;</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cape Town</td>
<td>2.26</td>
<td>1.00</td>
<td>15.61</td>
<td>18.90</td>
</tr>
<tr>
<td>Johannesburg</td>
<td>3.18</td>
<td>1.44</td>
<td>12.74</td>
<td>17.40</td>
</tr>
</tbody>
</table>

Costs of performing DST on culture (7H11 or MGIT) in Cape Town and Johannesburg laboratories

<table>
<thead>
<tr>
<th>Location</th>
<th>Decon.</th>
<th>Primary C.</th>
<th>Z/N</th>
<th>Auramine</th>
<th>DST, IR</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cape Town</td>
<td>2.26</td>
<td>7.82</td>
<td>1.57</td>
<td>1.00</td>
<td>12.01</td>
<td>24.66</td>
</tr>
<tr>
<td>Johannesburg</td>
<td>3.18</td>
<td>11.35</td>
<td>1.55</td>
<td>1.44</td>
<td>16.96</td>
<td>34.50</td>
</tr>
</tbody>
</table>

* does not include cost of identification (by Genotype Mycobacterium CM assay) which is approximately $17 per test.
Cost per valid test result
(Cape Town data)

• From sputum (n=591):
  – Total cost of MTBDR\textit{plus} testing from sputum = 591 \times $18.90 = $11,169.90
  – Proportion of interpretable results from sputum = 97.1\% (571/591)
• Cost per valid MTBDR\textit{plus} result from sputum = $19.56

• From culture (n=1104):
  – Total cost of testing from culture = 1104 \times $28.27 = $31,210.08
  – Proportion of interpretable results from culture = 94.5\% (1043/1104)
• Cost per valid MTBDR\textit{plus} result from culture = $29.92

• For MGIT culture + DST (n=1695),
  – Total cost of testing = 1695 \times $24.66 = $41,798.70
  – Proportion of interpretable results = 79.2\% (1343/1695)
• Cost per valid DST result = $31.12
Equipment costs

- $16,000 for manual line probe assay system (excluding equipment required for digestion-decontamination)
  - 12 samples per run
  - Includes thermal cycler, shaking platform and waterbath, heating block, sonicator, and microcentrifuge
  - Excludes other small equipment / consumables
    - Pipettes, microcentrifuge tubes, PCR tubes
- Automated GT Blot (Hain Lifescience)
  - 14 352 Euro
Conclusions

• Successful implementation in 2 high volume public health labs in South Africa
  – also in other settings: Samara, Nepal (Peru, Latvia)
  – Similar performance under operational conditions in high burden settings to published research studies (low incidence settings)

• High sensitivity and specificity
  – Some discrepancies cf. phenotypic DST, esp. INH

• Higher proportion of valid results than MGIT culture + DST in South Africa

• Equivalent performance from sputum and culture

• MTBDR\textit{plus} from sputum costs less than MGIT + DST (IR) in South Africa
FIND Hain Demonstration Projects


- Uganda
- South Africa
- Vietnam
- India
- Thailand
- Turkey
- Samara, RF
- China

* Sub-national coverage in India, China, Russia, Indonesia.

Legend:
- < 6%
- 6 - 20%
- 20 - 40%
- >40%
- No data

Ongoing 2008 implementation
WHO guidelines on implementation of line-probe assays for MDR TB (1)

- WHO Expert Committee meeting on 31 March
- Reviewed available data (including two meta-analyses) on line-probe assays
- Concluded: “…there is sufficient generalizable evidence to justify a recommendation on the use of line-probe assays for rapid screening for MER TB within country-specific settings.”
- STAG-TB recommendation to WHO on 23 June
- WHO issues policy guidelines on 1 July
TB program algorithm for MDR screening

TB suspect

Sputum AFB microscopy

AFB +

Rapid RIF test

RIF susceptible
- Treat drug susceptible TB

RIF resistant
- Refer for second-line DST

AFB -

MGIT+Capilia

Rapid RIF test

Negative
- Refer for second-line DST

Positive

RIF resistant
- Refer for second-line DST
MTB / Rif-resistance test

Workflow
• sputum
• simple 1-step external sample prep. procedure
• time-to-result < 2 h
• throughput: ≥ 16 tests / day / module
• no need for biosafety cabinet
• integrated controls
• true random access

Performance
• specific for MTB
• sensitivity better than smear, similar to culture
• detection of rif-resistance via rpoB gene

Product and system design
• test cartridges for GeneXpert System
• several GeneXpert modules can be combined in 1 workstation
• swap replacement of detection unit
• ~1 day technician training for non-mycobacteriologists
**rpoB Molecular Beacon Assay**

```
5' - GCCACGCGCCAGCTGAGCCAATTCATGGACGACAAAGAGATCCTGTTTACACAAACCTGCTGCGGCTGACCCGAGCAGCCTG - 3'
3' - CGTGGTGGTACGCTGGTTAAGTACCTGGTCTTGGGCGACAGCCCAACTGGTTGTCGGGGCTGACAGCCCGAC - 5'
```
Rifampin-resistant TB contains at least 1 *rpoB* mutations

Five-color PCR performed in a single well
Rifampin Resistant Sample

**Module Name**: A3

**Sample ID**: Buganda sputum.78

**Assay**: MTB Beta

**Assay Version**: 2

**Assay Type**: Research Use Only

**Reagent Lot ID**: 00502

**Cartridge S/N**: 0

**Expiration Date**: <None>

**Test Type**: Specimen

---

### Test and Analyte Result

<table>
<thead>
<tr>
<th>Analyte Name</th>
<th>Ct</th>
<th>EndPt</th>
<th>Analyte Result</th>
<th>Probe Check Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>Probe D</td>
<td>0.0</td>
<td>-1.0</td>
<td>NEG</td>
<td>PASS</td>
</tr>
<tr>
<td>Probe C</td>
<td>16.3</td>
<td>223.0</td>
<td>POS</td>
<td>PASS</td>
</tr>
<tr>
<td>Probe E</td>
<td>16.8</td>
<td>134.0</td>
<td>POS</td>
<td>PASS</td>
</tr>
<tr>
<td>Probe B</td>
<td>17.0</td>
<td>189.0</td>
<td>POS</td>
<td>PASS</td>
</tr>
<tr>
<td>Bg</td>
<td>27.2</td>
<td>233.0</td>
<td>NA</td>
<td>PASS</td>
</tr>
<tr>
<td>Probe A</td>
<td>15.7</td>
<td>131.0</td>
<td>POS</td>
<td>PASS</td>
</tr>
</tbody>
</table>
### Xpert TB development studies in Peru and Latvia

#### TB CASE DETECTION

|                | Xpert sensitivity in smear+, cul+ | Xpert sensitivity in smear-, cul+ | Xpert specificity in smear-, cul- *
<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>LATVIA</td>
<td>93.3% (14/15)</td>
<td>80% (12/15)</td>
<td>95.9% (71/74)</td>
</tr>
<tr>
<td>PERU</td>
<td>100% (99/99)</td>
<td>81.8% (9/11)</td>
<td>95.6% (151/158)</td>
</tr>
<tr>
<td>TOTAL 95% CI</td>
<td>99.1% (113/114) [95.2, 99.8]</td>
<td>80.8% (21/26) [62.1, 91.5]</td>
<td>95.7% (222/232) [93.2, 98.4]</td>
</tr>
</tbody>
</table>

*Follow-up of patients limited

#### RIF RESISTANCE DETECTION

<table>
<thead>
<tr>
<th></th>
<th>Xpert sensitivity in Rif resistant</th>
<th>Xpert specificity in Rif sensitive</th>
</tr>
</thead>
<tbody>
<tr>
<td>LATVIA</td>
<td>100% (8/8)</td>
<td>100% (18/18)</td>
</tr>
<tr>
<td>PERU</td>
<td>100% (14/14)</td>
<td>100% (94/94)</td>
</tr>
<tr>
<td>TOTAL 95% CI</td>
<td>100% (22/22) [85.1, 100]</td>
<td>100% (112/112) [96.7, 100]</td>
</tr>
</tbody>
</table>
Ugandan Clinical Sputum Samples

Out of 110 total samples tested in the GeneXpert
90 samples from Uganda retreatment patients suspected of TB
20 US sputum controls not suspected of TB:

- 73/74 culture positive $\rightarrow$ GX positive
  Sensitivity = 98.6%
- 20/20 culture negative TB not suspect $\rightarrow$ GX negative
  Specificity = 100%
- 6/16 culture negative TB suspect $\rightarrow$ GX positive.
  Sensitivity in culture negative TB clinical case = 37.5%
- 9/9 rifampin resistant by DST $\rightarrow$ rifampin resistant by GX
  Rifampin resistance “sensitivity” = 100%
- 1/65 rifampin susceptible by DST $\rightarrow$ rifampin resistant by GX
  Rifampin resistance specificity = 98.5% (sequence confirmation pending).
Sputum decontamination

- Vortex and stand for 15 minutes
- Fill tube to 15ml
- Centrifuge 15 min 3000g
- PBS
- NaOH-
- NALC
- 7H9
- PANTA
- OADC

MODS

Manual liquid culture and DST for <$2 material costs

- Culture and direct susceptibility testing
- rifampicin
- isoniazid
- drug free
- drug free

1  2  3  4
MODS – Implementation Issues

- Need for additional evaluation studies
  - Standardized procedures
  - Biosafety
  - Training
- Demonstration projects
  - Ease/feasibility of implementation
  - Technician acceptance
  - Costing data
  - Patient/public health impact
**Simplified TB diagnosis with the MDR-XDRTB Color Test**

Complexity of conventional sputum decontamination in reference labs

- 1. Liquefaction
- 2. Sample decanted
- 3. Decontamination NaOH
- 4. Vortex
- 5. Phosphate Buffer
- 6. Centrifugation
- 7. Decant
- 8. Re-suspend
- 9. Inoculation

**Simplicity of MDR-XDRTB COLOUR TEST for regional labs**

*Combined optimizations: single-step decontamination (Vasanthakuri et al 1987), microscopic observation of growth, direct susceptibility testing for MDRTB testing & XDRTB screening, selective culture media (Mitchison et al), color indication of culture positivity*

*Carlton Evans, Lima
**MDR-XDRTB Color Test for Regional Labs**

<table>
<thead>
<tr>
<th>Step</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Liquefaction &amp; decontamination in transport medium at room temperature</td>
</tr>
<tr>
<td>2</td>
<td>Direct application of 2 drops to selective thin layer agar for incubation in room air for MDRTB testing &amp; XDRTB screening</td>
</tr>
<tr>
<td>3</td>
<td>Color growth detection &amp; microscopy confirmation of morphology</td>
</tr>
</tbody>
</table>

- **Rifampicin**
- **Ciprofloxacin**
- **Isoniazid**

Biosafety similar to sputum microscopy because sputum is smeared directly onto the plate which is then permanently double-sealed until autoclaving.
MDR-XDRTB Color Test Performance (n=214)
Gold standard=culture positive in any test (n=84/214)

**TB diagnostic sensitivity**

![Bar graph showing sensitivity vs. any positive (+95%CI)]

- **ZN microscopy**: 51%
- **Centrifuge decontamination & thin layer agar Culture (TLA)**: 74%
- **MDR-XDRTB COLOR TEST**: 89%
- **Centrifuge decontamination & low-volume MODS**: 94%

**Concurrent Drug Susceptibility Testing**

<table>
<thead>
<tr>
<th>COLOUR TEST</th>
<th>Direct MODS</th>
<th></th>
<th>Indirect TEMA</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>MDR</strong></td>
<td>9</td>
<td>4</td>
<td>8</td>
<td>12</td>
</tr>
<tr>
<td><strong>not-MDR</strong></td>
<td>1</td>
<td>68</td>
<td>1</td>
<td>69</td>
</tr>
</tbody>
</table>

- **Total**: 10 | 71 | 81

**Color test had 2% contamination (all fungal)**
**Median time to positive result was 16 days**
Pros:
• Low-cost (<$1) – potentially sustainable & cost-effective
• Simple for human capacity-limited settings
• Safe – sealed solid agar suitable for basic regional labs
• Minimal risk of cross-contamination
• More sensitive & rapid than conventional solid culture
• Concurrent diagnosis & MDR-XDRTB testing

Cons:
• Requires 1-3 weeks
• Separate sample needed for microscopy

Priorities:
• Development studies to optimize test format
• Manufacturing partnerships with quality assurance
• Large-scale rigorous evaluation, including MDR & XDRTB
• Demonstration & impact evaluation
Benchmarking to commercial TB Antibody Kits?
Evaluation by WHO-TDR
ROC Analysis

Cunningham J. et al. 2005 (TDR/WHO)

Target: 80% sensitivity, >95% specificity
FIND’s approach to AG Rapid Detection

Do existing reagents work?

Suboptimal reagents

Suboptimal detection system

Suboptimal antigen

Improved reagents?

Better platform?

Novel antigens?

LAM in urine

New AB sets & cocktails

LFI ± reader

AG discovery
FIND’s approach to AB Rapid Detection

ANTIBODY ASSAY DEVELOPMENT

immPORT
Whole M.tb Proteome Arrays

ANTIGEN SELECTION

FIND CLINICAL SAMPLES

OTHER ANTIGEN SUPPLIERS

immPORT + PHRI + FIND

PRODUCT DEVELOPMENT

INDUSTRIAL PARTNER

INDUSTRIAL PARTNER

MANUFACTURING

Dx KIT

EVALUATION DEMONSTRATION

ACCESS

Timeline
Budget

May 08

May 09/10

May 11/12

Jun 11/12

FIND’s approach to AB Rapid Detection

ANTIBODY ASSAY DEVELOPMENT

immPORT
Whole M.tb Proteome Arrays

ANTIGEN SELECTION

FIND CLINICAL SAMPLES

OTHER ANTIGEN SUPPLIERS

immPORT + PHRI + FIND

PRODUCT DEVELOPMENT

INDUSTRIAL PARTNER

MANUFACTURING

EVALUATION DEMONSTRATION

ACCESS
Towards TB Serology: Anti-M.tbc Antibody Profiling

- Antigen array chip with ~4,000 proteins
- Whole M.tbc proteome screen to identify a set of diagnostic antigens for seroprofiling
- >1,000 sera selected for WPS (FIND sample repository)